

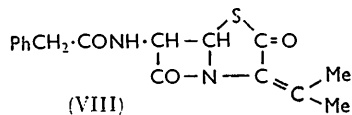
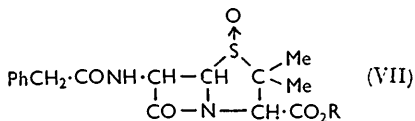
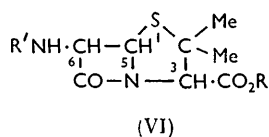
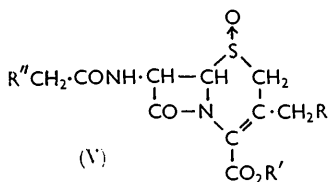
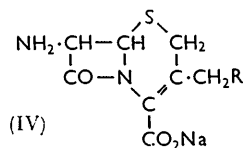
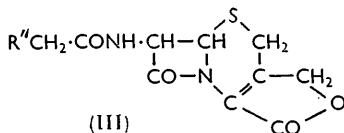
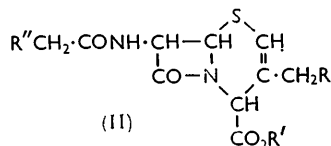
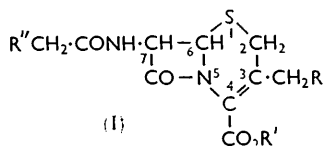
303. Cephalosporanic Acids. Part I. Infrared Absorption and Proton Magnetic Resonance Spectra of Cephalosporin and Penicillin Analogues

By G. F. H. GREEN, J. E. PAGE, and SUSAN E. STANIFORTH

The infrared absorption and the proton magnetic resonance spectra of 63 selected esters, salts, and free acids of assorted analogues and derivatives of cephalosporin and of penicillin have been recorded. The spectra are discussed, and structural assignments are made for each peak in the proton resonance spectra.

WE have recorded and have attempted to interpret the infrared absorption and the proton magnetic resonance (p.m.r.) spectra of fifty derivatives of cephalosporin and thirteen derivatives of penicillin. Although p.m.r. spectra of cephalosporin have been mentioned,¹ no detailed accounts of them have been published. The infrared, but not the p.m.r. spectra of penicillin analogues have been discussed previously.²

The cephalosporanate esters, which were soluble in bromoform and deuteriochloroform, gave infrared and p.m.r. spectra that were largely complementary and could be readily interpreted. The cephalosporanic acids and salts were insoluble in the normal solvents used for infrared spectroscopy and were therefore examined in the solid state as either Nujol mulls or alkali halide discs; the salts and free acids were, however, usually sufficiently



soluble in deuterium oxide and pyridine, respectively, to give good p.m.r. spectra. Since cephalosporanic acids and salts are frequently polymorphic, their solid-state infrared spectra often depend on the method of preparation, the purity of the sample, and the degree of drying, and are therefore less suitable for structural studies and identification purposes than are their p.m.r. spectra.

¹ (a) Abraham and Newton, *Biochem. J.*, 1961, **79**, 377; (b) Morin, Jackson, Mueller, Lavagnino, Scanlon, and Andrews, *J. Amer. Chem. Soc.*, 1963, **85**, 1896; (c) Stedman, Swered, and Hoover, *J. Medicin. Chem.*, 1964, **7**, 117.

² Bellamy, "The Infra-red Spectra of Complex Molecules," Methuen, London, 2nd edn., 1958, p. 214, and references there cited.

Infrared Spectra.—Bromoform solutions of the alkyl esters (I; $R' = \text{Me}$) of the substituted cephalosporanic acids and of the Δ^2 -analogues (II; $R' = \text{Me}$ or Et) named in Table 1 give well-defined infrared spectra (the spectra are not listed, but representative spectra have been deposited with the D.M.S. Scheme). The carbonyl stretching band for the β -lactam group of the Δ^3 -ester appears at a slightly higher value (1788—1782 cm^{-1}) than that of the corresponding Δ^2 -isomer (1774—1772 cm^{-1}); however, the frequencies of the ester carbonyl bands for a Δ^3 - (1736—1720 cm^{-1}) are, as might be expected, lower than those for a Δ^2 -isomer (1744—1740 cm^{-1}). The secondary amide bands for both the Δ^3 - (1680—1678 and 1505—1504 cm^{-1}) and the Δ^2 -isomers (1678—1676 and 1505—1504 cm^{-1}) are essentially the same. The β -lactam carbonyl band displacement is not unexpected in view of the known² shifts for carbonyl groups in fused and unfused β -lactam systems. The infrared spectra of bromoform solutions of γ -lactones derived from cephalosporanic acids are characterised by an intense band at about 1786 cm^{-1} and no absorption between 1750 and 1680 cm^{-1} .

The carbonyl bands for the cephalosporanic acids named in Table 2, when examined as Nujol mulls, show frequency shifts similar to those observed for the esters in bromoform solution; the β -lactam carbonyl bands for the Δ^3 - and Δ^2 -series appear at 1776—1764 and 1760—1756 cm^{-1} , respectively. The behaviour of the carboxyl carbonyl band for the Δ^3 -isomer, however, depends on the crystalline form of the sample, and the group gives rise either to two bands, at 1720—1708 and 1698—1692 cm^{-1} , or to one band at 1718—1701 cm^{-1} ; the Δ^2 -isomer gives a single carboxyl carbonyl band at 1735—1730 cm^{-1} . The secondary amide bands for the Δ^2 - (1678—1658 and 1544—1530 cm^{-1}) and Δ^3 -isomers (1675—1645 and 1548—1525 cm^{-1}) are again in the same range.

The β -lactam carbonyl groups of the sodium salts of the cephalosporanic acids and their Δ^2 -isomers (Table 3) absorb at lower frequencies (1768—1745 and 1750—1738 cm^{-1} , respectively) than do the corresponding free acids. The ionised carboxyl carbonyl and the secondary amide groups for both isomers give strong bands at 1627—1600 cm^{-1} and at 1667—1632 and 1542—1530 cm^{-1} , respectively. The acetate groups for the Δ^3 - and Δ^2 -isomers of both the sodium salts and the free acids absorb at 1753—1723 and 1260—1220 cm^{-1} .

The pyridinium derivatives (Table 4) exhibit β -lactam carbonyl bands in the range 1780—1754 cm^{-1} , the bands being at a higher frequency in well-dried than in wet samples; the undried samples also show a characteristic sharp band for water at 1670 cm^{-1} . The ionised carboxyl and secondary amide bands appear at 1626—1607 and at 1700—1646 and 1576—1527 cm^{-1} , respectively. 7-Aminocephalosporanic acid is exceptional in showing a β -lactam carbonyl band at 1806 and a zwitterionic carboxylic acid band at 1542 cm^{-1} . The penicillin salts and esters (Tables 5 and 6) gave the expected infrared absorption bands.²

*Proton Magnetic Resonance Spectra.**— β -Lactam ring protons. The p.m.r. spectra of Δ^2 - and Δ^3 -cephalosporanate esters in deuteriochloroform (Table 1) are characterised by a single-proton quartet centred at 4.18—4.41 τ and a single-proton doublet centred at 4.72—5.07 τ for the 7- and 6-protons, respectively, in the β -lactam ring. These peaks are a distinctive feature of the p.m.r. spectra of all cephalosporin analogues having an intact β -lactam ring, and can be used to distinguish cephalosporin from penicillin analogues.

In cephalosporanate esters (I; $R' = \text{Me}$), the β -lactam quartets and doublets are centred at 4.18—4.20 and 5.02—5.07 τ , respectively (*i.e.*, 0.89—0.84 τ units apart), and in Δ^2 -esters (II; $R' = \text{Me}$ or Et) at 4.33—4.41 and at 4.72—4.79 τ , respectively (*i.e.*, 0.41—0.38 τ units apart) (Table 1). The 7-proton quartet consists of a pair of doublets and is attributed to coupling with the 6-proton on the one side ($J = 4$ —5 c./sec.) and with the imino-proton on the other ($J = 8$ —9 c./sec.). This assignment is confirmed by deuteration, when the quartet collapses to a doublet with the same coupling constant (4—5 c./sec.) as

* In the Tables the following abbreviations are used: d, doublet; t, triplet; q, quadruplet; m, multiplet; n.o., line not observed, obscured by solvent absorption.

TABLE I
Proton-resonance lines (τ values) for esters and γ -lactone of cephalosporanic acids in
CDCl₃ solution (J values in c./sec. in parentheses)

Compound	R	R'	R''	Ref.	2-H	3-CH ₂	R	4-H	R'	6-H	7-H	7-NH	R''CH ₂
(I) OH	OH	Me	Ph	a	6-21d (18) 6-60d (18)	5-51d (13) 6-02d (13)	7-28	—	6-15	5-07d (4-5)	4-18q* (4-5; 9)	3-64d (9)	2-69, 6-15
(I) OAc	OAc	Me	Ph	b	6-39d (19) 6-75d (19)	4-85d (13) 5-21d (13)	7-92	—	6-14	5-04d (5)	4-18q* (5; 9)	3-68d (9)	2-67, 6-35
(I) S-CS-NMe ₂	S-CS-NMe ₂	Me	Ph	b	6-43d (18) 6-77d (18)	5-18d (14) 5-65d (14)	6-42	—	6-14	5-05d (4-5)	4-20q* (4-5; 9)	3-85d (9)	2-67, 6-37
(I) N ₃	N ₃	Me	Ph	b	6-40d (18) 6-69d (18)	5-59d (14) 6-00d (14)	—	—	6-13	5-04d (4-8)	4-18q* (4-8; 9)	3-72d (9)	2-65, 6-35
(I) OAc	OAc	Me	2-Thienyl	b	6-38d (18) 6-72d (18)	4-82d (13) 5-22d (13)	7-92	—	6-14	5-04d (5)	4-18q* (5; 9)	3-32d (9)	2-72t (3-5) 3-03d (3-5) 6-14
(I) OAc	OAc	Me	Pr ^a	b	6-34d (18) 6-70d (18)	4-82d (13) 5-18d (13)	7-91	—	6-13	5-02d (5)	4-18q* (5; 8-5)	3-53d (8-5)	7-72t (7) 8-23-8-81m 9-09t (7)
(II) OH	OH	Me	Ph	c	3-75	5-82d (3)	7-50	4-94	6-21	4-79d (4)	4-41q* (4; 9)	3-27d (9)	2-70, 6-37
(II) OAc	OAc	Me	Ph	c	3-58	5-36d (3)	7-92	4-99	6-18	4-74d (4)	4-35q* (4; 8)	3-68d (8)	2-65, 6-34
(II) OAc	OAc	Et	Ph	c	3-59	5-35d (3)	7-93	5-00	5-75q (7-5) 8-70t (7-5)	4-72d (4)	4-34q* (4; 9)	3-73d (9)	2-65, 6-33
(II) OAc	OAc	Et	2-Thienyl	c	3-62	5-36d (3)	7-93	5-02	5-76q (7-5) 8-70t (7-5)	4-74d (4)	4-33q* (4; 8-5)	3-52d (8-5)	2-73t (3-5) 3-02d (3-5) 6-14
(III) †	—	—	Ph	b	6-06d (19) 6-42d (19)	4-85	—	—	—	4-72d (5)	3-95q* (5; 9)	2-72d (9)	2-60, 6-10

* Two doublets with the J values given. † In trifluoroacetic acid. (a) Chauvette, Flynn, Jackson, Lavagnino, Morin, Mueller, Pioch, Roeske, Ryan, Spencer, and van Heyningen, *J. Amer. Chem. Soc.*, 1962, **84**, 3401; (b) Cocker, Cowley, Cox, Eardley, Gregory, Lazenby, Long, Somerfield, and Sly, *J.*, in the press; (c) Cocker, Eardley, Gregory, Hall, and Long, unpublished results.

TABLE 2
Proton-resonance lines (τ values) for cephalosporanic acids in pyridine solution
(J values in c./sec. in parentheses)

Compound	R	R'	R''	Ref.	2-H	3-CH ₂	R	4-H	6-H	7-H	R''CH ₂
(I) H	H	H	Ph	a	6-45d (18), 6-85d (18)	7-87	7-87	—	4-78d (4-5)	3-72q* (4-5; 8-5)	n.o., 6-13
(I) OAc	OAc	H	Ph	b	6-18d (18), 6-53d (18)	4-49d (13), 4-87d (13)	8-00	—	4-72d (4-5)	3-66q* (4-6; 8-4)	n.o., 6-13
(I) SEz	SEz	H	Ph	c	6-07d (18), 6-46d (18)	5-25d (13-5), 5-60d (13-5)	n.o.	—	4-69d (5)	3-62q* (5; 8)	n.o., 6-10
(I) 3-Indolyl	3-Indolyl	H	Ph	c	6-32d (18), 6-65d (18)	5-43d (15), 5-88d (15)	n.o.	—	4-76d (4-5)	3-72q* (4-5; 8)	n.o., 6-13
(I) N-Methyl-3-indolyl	N-Methyl-3-indolyl	H	Ph	c	5-98d (18), 6-33d (18)	5-54d (15), 5-98d (15)	n.o.	—	4-75d (4-5)	3-75q* (4-5; 8-5)	n.o., 6-49
(I) OAc	OAc	H	2-Thienyl	c	6-18d (18), 6-53d (18)	4-50d (13), 4-88d (13)	8-02	—	4-73d (5)	3-67q* (5; 8-2)	n.o., 5-90
(I) SEz	SEz	H	2-Thienyl	c	6-08d (18), 6-46d (18)	5-28d (13), 5-60d (13)	n.o.	—	4-70d (4-8)	3-67q* (4-8; 8)	n.o., 5-90
(I) OAc	OAc	H	2-Thienyl	d	6-12d (18), 6-50d (18)	4-92d (13-5), 5-27d (13-5)	n.o.	—	4-66d (4-8)	3-64q* (4-8; 8-5)	n.o., 5-90
(II) OAc	OAc	H	Ph	d	3-40d (0-8)	4-86d (13), 5-13d (13)	8-08	4-53d (1)	4-24d (4)	3-89q* (4; 8)	n.o., 6-10
(II) OAc	OAc	Me	Ph	d	3-35d (1-5)	5-22	8-07	4-57d (2)	4-42d (4)	3-88q* (4; 8-2)	n.o., 6-10
(II) OAc	OAc	H	2-Thienyl	d	3-40d (1)	4-86d (13), 5-15d (13)	8-06	4-53d (1)	4-22d (4)	3-90q* (4; 8)	n.o., 5-86

* Two doublets with the J values given. (a) Ref. 1c. (b) Loder, Newton, and Abraham, *Biochem. J.*, 1961, **79**, 408. (c) Ref. (b), Table 1. (d) Ref. (c), Table 1.

TABLE 3
Proton-resonance lines (τ values) for salts of cephalosporanic acids in D₂O solution
(J values in c./sec. in parentheses)

Compound		R'	R''	Ref.	2-H	3-CH ₂	R	4-H	6-H	7-H	R''CH ₂
(IV)	OAc	—	—	<i>a</i>	6-22d (18) 6-60d (18)	4-99d (13) 5-26d (13)	7-82	—	4-87d (4-5)	50% 4-47d (4-5) 50% 5-17d (4-5)	—
(I)	OH	Na	Ph	<i>b</i>	6-26d (18) 6-63d (18)	5-69	—	—	4-87d (4-6)	4-35d (4-6)	2-58 6-26
(I)	OAc	Na	Ph	<i>c</i>	6-30d (18) 6-68d (18)	5-04d (13) 5-30d (13)	7-85	—	4-88d (4-5)	4-33d (4-5)	2-58 6-28
(I)	SEt	Na	Ph	<i>c</i>	6-40d (16) 6-43d (14)	6-07d (14) 6-43d (14)	7-46q (7-3) 8-77t (7-3)	—	4-85d (4-5)	4-38d (4-5)	2-55 6-25
(I)	4-Methyl-2-pyr- imidinythio	Na	Ph	<i>c</i>	6-43d (18) 6-83d (18)	5-53d (13) 6-07d (13)	1-70d (5-5) 3-03d (5-5) 7-63	—	5-06d (4-5)	4-40d (4-5)	2-82 6-36
(I)	N ₃	Na	Ph	<i>c</i>	6-31d (18) 6-71d (18)	5-88d (1)	—	—	4-89d (4-5)	4-36d (4-5)	2-60 6-30
(I)	OAc	Na	2-Thienyl	<i>c</i>	6-29d (18) 6-70d (18)	4-99d (13) 5-26d (13)	7-83	—	4-86d (4-5)	4-30d (4-5)	2-58t (3-5) 2-90d (3-5)
(I)	S-CS-NMe ₃	Na	2-Thienyl	<i>c</i>	6-29d (18) 6-67d (18)	5-35d (13) 5-72d (13)	6-50 6-60	—	4-94d (4-5)	4-36d (4-5)	6-05 2-99d (3) 6-10
(I)	OAc	Na	MeS	<i>c</i>	6-23d (18) 6-60d (18)	4-99d (13) 5-25d (13)	7-83	—	4-80d (4-6)	4-28d (4-6)	6-61 7-75
(I)	OAc	Na	[CH ₂] ₂ CH(NH ₂)-CO ₂ Na	<i>d</i>	6-17d (17) 6-57d (17)	4-95d (13) 5-23d (13)	7-83	—	4-83d (4-5)	4-31d (4-5)	6-19t (6) 7-52t (6)
(I)	N ₃	Na	Pr ^a	<i>c</i>	6-22d (18) 6-62d (18)	5-83d (2)	—	—	4-82d (4-6)	4-32d (4-6)	8-04-8-30m 7-60t (6) 8-0-8-8m
(I)	N ₃	Na	SBz	<i>c</i>	6-33d (18) 6-75d (18)	5-71d (14) 5-97d (14)	—	—	4-85d (4-5)	4-35d (4-5)	9-06t (6) 2-67 6-20
(II)	OH	Na	Ph	<i>e</i>	3-65d (1-5) 3-52d (2)	5-75d (1) 5-17d (1)	7-86	5-11d (1-5)	4-69d (4)	4-57d (4)	6-75
(II)	OAc	Na	Ph	<i>e</i>	3-48d (2) 3-55	5-13d (2) 5-23d (2)	7-82 7-82	5-17d (1)	4-71d (4)	4-58d (4)	2-55, 6-26 2-60, 6-28
(II)	OAc	NH ₄	Ph	<i>e</i>	3-48d (2)	5-13d (2)	7-82	5-17	4-66d (4)	4-54d (4)	2-56, 6-23
(II)	O-CO-Pr ^a	Na	Ph	<i>e</i>	3-55	5-23d (2)	7-60t (7) 8-37m	5-24	4-70d (4)	4-58d (4)	2-60 6-29
(II)	OAc	Na	2-Thienyl	<i>e</i>	3-52d (2)	5-16d (2)	7-88	5-16	4-68d (4)	4-57d (4)	2-60t (3-5) 2-93d (3-5) 6-08

TABLE 3 (Continued)

(II) OAc	Na	Et	e	3-45d (2)	5-13d (2)	7-83	5-13d (1-5)	4-63d (4)	4-52d (4)	7-62t (7) 8-32m 9-04t (7)
(V) OAc	Na	Ph	c	6-06d (18-5) 6-49d (18-5)	4-87d (13) 5-29d (13)	7-85	—	5-02d (5)	4-10d (5)	2-56 6-22
(V) OAc	Na	2-Thienyl	c	6-05d (18) 6-50d (18)	4-90d (13) 5-33d (13)	7-89	—	5-12d (4-6)	4-12d (4-6)	2-57t (3-5) 2-92d (3-5)
(II) OAc	Pyr- idine	Ph	e	3-56d (1-5)	5-28d (1)	7-89	5-14 d(1-5)	4-71d (4-2)	4-58d (4-2)	6-05 2-62
(II) OAc	Pyr- idine	2-Thienyl	e	3-53d (2)	5-27d (1)	7-89	5-17d (2)	4-70d (4)	4-58d (4)	6-32 2-63t (3-2) 2-95d (3-2) 6-09

(a) Morin, Jackson, Flynn, and Roeske, *J. Amer. Chem. Soc.*, 1962, **84**, 3400. (b) Ref. (a), Table 1. (c) Ref. (b), Table 1. (d) Newton and Abraham, *Biochem. J.*, 1966, **62**, 651; (e) Ref. (c), Table 1.

TABLE 4

Proton-resonance lines (τ values) for pyridinium derivatives of cephalosporamic acids in D_2O solution (J values in c./sec. in parentheses)

Compound	R	R'	R''	Ref.	2-H	3-CH ₂	R	4-H	6-H	7-H	R''CH ₂
(I) Pyridinium	—	Ph	Ph	a	6-40d (18) 6-93d (18)	4-39d (15) 4-73d (15)	1-02d (7) 1-38t (7) 1-90t (7)	—	4-88d (5)	4-34d (5)	2-67 6-35
(I) Pyridinium	—	2-Thienyl	2-Thienyl	a	6-38d (18) 6-90d (18)	4-40d (15) 4-70d (15)	1-02d (7) 1-38t (7) 1-88t (7)	—	4-88d (5)	4-33d (5)	2-68t (3-5) 3-00d (3-5) 6-13
(I) Pyridinium chloride	H	2-Thienyl	2-Thienyl	a	6-29d (18-5) 6-81d (18-5)	4-20d (15) 4-66d (15)	1-05d (7) 1-37t (7) 1-90t (7)	—	4-85d (5)	4-30d (5)	3-09d (3-5) 6-13
(I) Pyridinium lactate *	H	2-Thienyl	2-Thienyl	a	6-37d (18) 6-89d (18)	4-37d (14) 4-70d (14)	1-02d (7) 1-38t (7) 1-88t (7)	—	4-87d (5)	4-34d (5)	2-69t (3-5) 3-02d (3-5) 6-12
(II) Pyridinium	—	Ph	Ph	b	3-23d (1)	4-67	1-10d (7) 1-40t (7)	5-42d (1)	4-60	4-56d (3)	2-60 6-28
(II) Pyridinium	—	2-Thienyl	2-Thienyl	b	3-20d (1)	4-65	1-92t (7) 1-11d (7) 1-37t (7) 1-88t (7)	5-40d (1)	4-58	4-52d (3)	2-59t (3-5) 2-93d (3-5) 6-05

* Not isolated in pure form. Lactate resonances at 5-60q (7) and 8-56d (7). (a) Eardley, Kennedy, Long, and Stocker, unpublished results. (b) Ref. (c), Table 1.

TABLE 5
Proton-resonance lines (τ values) for penicillin salts (VI) in D₂O solution (J values in c./sec. in parentheses)

R	R'	Ref.	2-Me ₂	3-H	5-H	6-H	R ¹
Na	H	<i>a</i>	8.33, 8.43	5.76	4.46d (4.0)	5.36d (4.0)	—
Na	CO·CH ₂ ·Ph	<i>b</i>	8.39, 8.46	5.71	4.56d (4.0)	4.44d (4.0)	2.60, 6.30
K	CO·CH ₂ ·Ph	<i>b</i>	8.40, 8.47	5.72	4.55d (4.0)	4.44d (4.0)	2.60, 6.30
Na	CO·CH ₂ ·OPh	<i>c</i>	8.50	5.74	4.50d (4.0)	4.44d (4.0)	2.60—3.72m, 5.48
Na	CO·2,6-C ₆ H ₃ (OMe) ₂	<i>d</i>	8.33, 8.42	5.72	4.35d (4.0)	4.27d (4.0)	2.54q * (8.5; 7.5), 3.22d (8.5), 6.13
H	CO·CH(NH ₂)·Ph	<i>e</i>	8.50, 8.57	5.77	4.49	4.49	2.51, 5.08
Na	3-Phenyl-5-methyl-4-isoxazoly	<i>f</i>	8.54, 8.57	5.85	4.49d (4.0)	4.41d (4.0)	2.50, 7.42

* Two doublets with the J values given. (*a*) Batchelor, Doyle, Naylor, and Rolinson, *Nature*, 1959, 183, 257. (*b*) Clarke, Johnson, and Robinson. "The Chemistry of Penicillin," Princeton University Press, Princeton, 1949. (*c*) Behrens, Corse, Edwards, Garrison, Jones, Soper, van Abeele, and Whitehead, *J. Biol. Chem.*, 1948, 175, 793. (*d*) Batchelor, Chain, Rolinson, Stevens, and Wood, *Lancet*, 1960, ii, 564. (*e*) Rolinson and Stevens, *Brit. Med. J.*, 1961, ii, 191. (*f*) Doyle, Long, Naylor, and Stove, *Nature*, 1961, 192, 1183.

TABLE 6

Proton-resonance lines (τ values) for benzylpenicillin esters (VI; R' = CO·CH₂·Ph) and derivatives in CDCl₃ solution (J values in c./sec. in parentheses)

Compound	R	Ref.	2-Me ₂	3-H	CO ₂ R	5-H	6-H	NH	PhCH ₃
(VI) H	H	<i>a</i>	8.48, 8.55	5.62	—0.57	4.48 4.50d (4) †	4.30d (4), 4.51d (4) *	3.65d (8.5)	2.66, 6.33
(VI) <i>p</i> -C ₆ H ₄ Cl	<i>b</i>	<i>b</i>	8.38, 8.46	5.41	2.65d (9.5), 2.98d (9.5)	4.43 4.47d (4) †	4.25d (4), 4.47d (4) *	3.79d (8)	2.68, 6.35
(VI) <i>p</i> -C ₆ H ₄ Me	<i>b</i>	<i>b</i>	8.37, 8.46	5.42	2.83d (8.5), 3.06d (8.5), 7.65	4.43 4.48d (4) †	4.27d (4), 4.47d (4) *	3.77d (8)	2.69, 6.36
(VI) <i>p</i> -C ₆ H ₄ NMe ₂	<i>b</i>	<i>b</i>	8.39, 8.48	5.44	3.06d (9.5), 3.35d (9.5), 7.09	4.43 4.48d (4) †	4.28d (4), 4.47d (4) *	3.76d (8)	2.69, 6.37
(VII) CH ₂ Ph	<i>a</i>	<i>a</i>	8.38, 8.97	5.37	2.73, 4.68d (12), 4.92d (12)	5.05d (4.5)	4.02q † (4.5; 10)	2.92d (10)	2.65, 6.44
(VIII) —	<i>c</i>	<i>c</i>	7.82, 7.94	—	—	4.49d (4.0)	4.16q † (4; 8.5)	3.42d (8.5)	2.71, 6.39

* Equal intensity. † On deuteration. ‡ Two doublets with the J values given. (*a*) Ref. (*b*), Table 5. (*b*) Barnden, Evans, Hamlet, Hems, Jansen, Trevett, and Webb, *J.*, 1953, 3733. (*c*) Wolfe, Godfrey, Holdrege, and Perron, *J. Amer. Chem. Soc.*, 1963, 85, 643.

TABLE 7

Proton-resonance lines (τ values) for methyl 7-phenylacetamidoccephalosporanate
(I; R = AcO, R' = Me, R'' = Ph) in different solvents (J values in c./sec. in parentheses)

Solvent	2-H	3-CH ₃	Ac	CO ₂ Me	6-H	7-H	N-H	PhCH ₂
CDCl ₂	6-39d (19), 6-75d (19)	4-85d (13), 5-21d (13)	7-92	6-14	5-04d (5)	4-18q * (4-5; 9)	3-68d (9)	2-67, 6-35
Acetone	6-25d (18-5), 6-60d (18-5)	4-93d (13), 5-27d (13)	n.o.	6-18	4-87d (5)	4-17q * (5; 8-7)	2-07d (8-7)	2-70, 6-35
MeCN	6-33d (18-5), 6-68d (18-5)	5-02d (13), 5-33d (13)	n.o.	6-20	4-99d (5)	4-27q * (5; 8-5)	2-75d (8-5)	2-72, 6-45
PhCN	6-26d (18), 6-64d (18)	4-78d (13-5), 5-16d (13-5)	7-96	6-18	4-99d (5)	3-99q * (5; 8-5)	n.o.	n.o., 6-28
H ₂ CO ₃ H	6-15d (18-5), 6-52d (18-5)	4-76d (13-5), 5-05d (13-5)	7-79	6-03	4-78d (4-8)	4-10q * (4-8; 8-3)	2-02d (8-3)	2-62, 6-13
CF ₃ -CO ₂ H	6-20d (19), 6-57d (19)	4-66d (13-5), 4-95d (13-5)	7-73	5-96	4-80d (4-5)	4-10q * (4-5; 8-5)	2-67d (8-5)	2-62, 6-10
Me ₂ NCOH	n.o.	4-94d (13-5), 5-26d (13-5)	7-93	n.o.	4-80d (5)	4-17q * (5; 8-5)	2-65d (8-5)	2-69, 6-30
Me ₂ NAC	n.o.	4-98d (13), 5-27d (13)	7-98	6-16	4-78d (5)	4-17q * (4-8; 8-5)	2-35d (8-5)	2-73, n.o.
Dioxan	n.o.	4-75d (13-5), 5-12d (13-5)	8-03	6-20	4-75d (4-5)	3-65q * (5; 8-5)	n.o.	2-67, 6-15
Pyridine	6-22d (18-5), 6-56d (18-5)	5-02d (13), 5-35d (13)	n.o.	6-21	4-88d (4-8)	4-28q * (4-8; 8-5)	0-93d (8-5)	2-72, 6-43
Me ₂ SO	6-28d (18), n.o.							

* Two doublets with the J values given.

TABLE 8

Proton-resonance lines (τ values) for 7-phenylacetamidoccephalosporanic acid
(I; R = AcO, R' = H, R'' = Ph) in different solvents (J values in c./sec. in parentheses)

Solvent	2-H	3-CH ₃	Ac	6-H	7-H	NH	PhCH ₂
Acetone	6-28d (18-5), 6-58d (18-5)	4-89d (13-5), 5-20d (13-5)	n.o.	4-89d (5)	4-17q * (5; 8-5)	2-01d (8-5)	2-68, 6-32
MeCN	6-32d (18), 6-66d (18)	4-95d (13), 5-28d (13)	n.o.	4-98d (5)	4-26q * (5; 8-5)	2-70d (8-5)	2-70, 6-43
H ₂ CO ₃ H	6-14d (19), 6-49d (19)	4-68d (14), 4-98d (14)	7-79	4-76d (5)	4-10q * (5; 8-5)	2-00d (8)	2-61, 6-14
CF ₃ -CO ₂ H	6-20d (18), 6-54d (18)	4-56d (14), 4-86d (14)	7-73	4-79d (4-5)	4-12q * (4-5; 8-5)	2-69d (8-5)	2-61, 6-11
Pyridine	6-18d (18), 6-53d (18)	4-49d (13), 4-87d (13)	8-00	4-72d (4-5)	3-66q * (4-5; 8-4)	n.o.	n.o., 6-13

* Two doublets with the J values given.

that shown by the 6-proton doublet; the imino-proton doublet at 3.27—3.85 τ disappears. The position of the imino-proton doublet varies widely from one analogue to another (Table 1) and also depends on the nature of the solvent (Table 7), being at a minimum value for solutions in dimethyl sulphoxide.

The 6- and 7-protons of Δ^3 -cephalosporanic acids in pyridine (Table 2) yield a doublet centred at 4.66—4.78 and a quartet centred at 3.62—3.75 τ , respectively; the quartet reduces to a doublet on deuteration. Solvent absorption obscures the imino-proton peaks. The 6- and 7-proton peaks for the corresponding Δ^2 -acids are centred at 4.22—4.24 and 3.89—3.90 τ , respectively.

The imino-protons for sodium cephalosporanates in deuterium oxide undergo exchange with deuterium so that only the doublets for the 6- and 7-protons appear (Table 3). In Δ^3 -salts (I; R' = Na) the 7- and 6-proton doublets are centred at 4.28—4.40 and 4.80—5.06 τ , respectively, and in Δ^2 -salts (II; R' = Na) at 4.52—4.58 and 4.63—4.71 τ , respectively; the coupling constants are 4.0—4.6 c./sec. Sodium 7-aminocephalosporanate (IV; R = AcO) behaves anomalously. The doublet centred at 4.47 τ , which is associated with the 7-proton, has only half the expected intensity; a second doublet with an intensity corresponding to that for half a proton is centred at 5.19 τ . If the solution is kept at room temperature, the intensity of the 4.47 τ doublet decreases and that of the 5.19 τ doublet increases. The 6-proton doublet centred at 4.87 τ is of normal intensity.

Pyridinium analogues of cephalosporin (Table 4) in deuterium oxide show characteristic β -lactam peaks that can be used to distinguish Δ^2 - from Δ^3 -isomers.

Our measurements on methyl 7-phenylacetamidocephalosporanate (I; R = AcO, R' = Me, R'' = Ph) in a wide range of solvents (Table 7) show that the β -lactam proton resonances and the coupling constants differ slightly from one solvent to another; this effect is also shown by 7-phenylacetamidocephalosporanic acid (Table 8).

The centres of the β -lactam doublets for 6-acylamidopenicillanic acid salts in deuterium oxide (Table 5) are much closer together (0—0.12 τ units apart) than are those for salts of Δ^3 - (0.47—0.66 τ units apart) and slightly closer than those for Δ^2 -cephalosporanates (0.11—0.13 τ units apart) (Table 3); these displacements are large enough to be of value in distinguishing a penicillin from a Δ^3 -cephalosporin ring system. Sodium 6-amino-penicillanate is exceptional in that the doublets for the 5- and 6-protons are centred at 4.46 and 5.36 τ , respectively (0.90 τ units apart).

Oxidation of the sulphur atom to sulphoxide changes the environment of the β -lactam protons in both the cephalosporin and the penicillin molecules and increases the chemical shift between the two β -lactam protons. The centres of the β -lactam doublets for salts of cephalosporin sulphoxides (V) and for the ester of penicillin sulphoxide (VII) are 0.92—1.00 and 1.03 τ units apart, respectively (Tables 3 and 6).

The β -lactam peaks for penicillin esters in deuteriochloroform (Table 6) and for pyridinium analogues of Δ^2 -cephalosporanic acids (II; R = pyridinium) in deuterium oxide (Table 4) are anomalous. The pattern for the penicillin esters consists of a sharp singlet at 4.43—4.48 τ for the 5-proton and a pair of doublets of equal intensity centred at 4.25—4.30 and 4.47—4.51 τ ($J = 4$ c./sec.) for the 6-proton. The imino-proton gives a doublet at 3.65—3.79 τ ($J = 8.0$ — 8.5 c./sec.), which disappears on deuteration, the 5- and 6-proton peaks rearranging to give two doublets centred at 4.47—4.50 and 4.33—4.37 τ ($J = 4$ c./sec.), respectively (*i.e.*, 0.12—0.15 τ units apart). Penicillin sulphoxides (VII) and anhydropenicillins (VIII) give the normal pattern of β -lactam peaks (Table 6).

Thiazinyl ring protons. The 2-methylene group of a methyl cephalosporanate (I; R = AcO, R' = Me), which has two protons in different chemical environments, gives rise in deuteriochloroform solution to two single-proton doublets centred at 6.34—6.39 and 6.70—6.75 τ , the coupling constants being about 18 c./sec. (Table 1). The 2- and 4-protons in the corresponding Δ^2 -esters (II; R = AcO, R' = Me or Et) yield broad single-proton singlets at 3.58—3.62 and 4.99—5.02 τ , respectively. This band pattern provides direct evidence for the double-bond shift and is the most characteristic spectroscopic difference

between the two isomers. Deacetylation displaces the centres of the 2-methylene doublets for the Δ^3 -isomer (I; R = OH, R' = Me) to 6.21 and 6.60 τ and moves the 2- and 4-proton peaks for the Δ^2 -isomer (II; R = OH, R' = Me) to 3.75 and 4.94 τ , respectively.

Exocyclic methylene protons. The methylene group exocyclic to the thiazinyl ring in methyl cephalosporanates (I; R = AcO, R' = Me), but not in the corresponding Δ^2 -isomers (II; R = AcO, R' = Me) (Table 1), behaves unexpectedly in showing two single-proton doublets centred at 4.82—4.85 and 5.18—5.22 τ ($J = 13$ c./sec.); the positions of the peaks depend on the solvent and on the nature of the groups substituted on the methylene groups. Thus, the exocyclic methylene peaks for methyl 7-phenylacetamidocephalosporanate (I; R = AcO, R' = Me, R'' = Ph) in acid solvents, such as trifluoroacetic acid, are centred at lower magnetic field strengths than those for the ester in deuteriochloroform solution; the coupling constant is unchanged at 13.0—13.5 c./sec. (Table 7). This splitting of the peaks for the exocyclic methylene group, which shows that the methylene protons are non-equivalent and that the acetoxy-methylene group is unable to rotate freely, is similar to that shown by the 21-methylene protons of 21-acetoxy-17-hydroxypregnan-20-ones³ and the 2,2'-methylene protons of DL-6,6'-diethyl-2,2'-bishydroxymethylbiphenyl.^{4*} The exocyclic methylene protons of sodium salts (Table 3) and of pyridinium analogues (Table 4) of acetoxy- Δ^3 -isomers in deuterium oxide show similar pairs of doublets centred at 4.95—5.04 and 5.23—5.30 τ and at 4.20—4.40 and 4.66—4.73 τ , respectively; the corresponding sodium salts and pyridinium analogues of acetoxy- Δ^2 -isomers, however, merely yield broad singlets for these protons. Replacement of the acetoxy-group of sodium cephalosporanates by hydroxyl or azido-groups also leads to the appearance of a broad singlet (Table 3). Splitting occurs, however, if the 7-phenylacetamido-side-chain of an azido-analogue is replaced by a benzylthioacetamido-group. The exocyclic methylene protons of cephalosporamic acids (I; R' = H) in pyridine, acetone, acetonitrile, formic acid, and trifluoroacetic acid (Tables 2 and 8) give rise to pairs of doublets; this arrangement, with doublets centred at 4.86 and 5.13—5.15 τ ($J = 13$ c./sec.), is also shown, rather unexpectedly, by Δ^2 -acids (II; R' = H) in pyridine solution (Table 2), suggesting that pyridine forms a salt and prevents free rotation of the acetoxy-methylene group. The chemical shift between the centres of the doublets for methyl 7-phenylacetamidocephalosporanate ranges from about 0.38 τ for a benzonitrile to 0.29 τ for a dioxan solution (Table 7; see Snyder⁵).

The effect of temperature on the peaks for the exocyclic methylene protons was studied by recording the p.m.r. spectra of a dimethyl sulphoxide solution of methyl 7-phenylacetamidocephalosporanate (I; R = AcO, R' = Me, R'' = Ph) at various temperatures up to 160° (cf. Hammond and Neuman⁶); there were no significant changes in the positions and coupling constants of the exocyclic methylene peaks. The exocyclic methylene and the β -lactam proton peaks for this ester overlap in the p.m.r. spectra of solutions in many high-boiling solvents, but are well separated in the spectra of solutions in dimethyl sulphoxide. Measurements at temperatures up to 180° did not reveal any changes in the peaks for the 21-methylene protons of 3,21-diacetoxy-17-hydroxy-5 α -pregnane-11,20-dione in benzonitrile solution.

Our p.m.r. evidence for the non-equivalence of the exocyclic methylene protons is supported by X-ray measurements on sodium cephalosporin (I; R = AcO, R' = Na, R'' = [CH₂]₂·CH(NH₂)·CO₂Na). Hodgkin and Maslen⁷ found that C-2, C-3, C-4, N-5, and

* Since this Paper was submitted for publication Whitesides, Holtz, and Roberts (*J. Amer. Chem. Soc.*, 1964, **86**, 2628; see also Nair and Roberts³) have pointed out that steric hindrance is not essential for the protons of a methylene group to show non-equivalence; it is only necessary for the methylene group to rotate in an asymmetric environment.

³ Shoolery and Rogers, *J. Amer. Chem. Soc.*, 1958, **80**, 5121; see also Nair and Roberts, *ibid.*, 1957, **79**, 4565.

⁴ Meyer and Meyer, *J. Amer. Chem. Soc.*, 1963, **85**, 2170.

⁵ Snyder, *J. Amer. Chem. Soc.*, 1963, **85**, 2624.

⁶ Hammond and Neuman, *J. Phys. Chem.*, 1963, **67**, 1655.

⁷ Hodgkin and Maslen, *Biochem. J.*, 1961, **79**, 393.

the carbon atoms of the exocyclic methylene, and the 4-carboxyl groups lie in a plane; the sulphur atom is about 0.6 Å above and C-6 about 0.6 Å below the plane. A Dreiding model shows that, with this stereochemical arrangement, the 3-acetoxymethyl- and the 4-carboxyl-groups would interact sterically, thus preventing free rotation of the acetoxy-methylene group and giving rise to a pair of doublets in the p.m.r. spectrum. A similar effect would be expected for other cephalosporanic acids, salts, and esters (cf. Tables 1—4). Replacement of the acetoxy-group by hydroxyl or azido (I; R = OH or N₃) reduces the chance of interaction, thus accounting for the broad singlet in the p.m.r. spectra of sodium salts of these acids (Table 3). The appearance of a pair of doublets in the spectrum of the benzylthioacetamido-analogue (I; R = N₃, R' = Na, R'' = S·CH₂·Ph) is explained by the benzylthioacetamido-side-chain bending over and interacting with the azidomethylene group.

In Δ²-cephalosporanic esters and salts (II; R' = Me or Na), side-chain interaction and the appearance of a pair of doublets for the exocyclic methylene group would be expected if C-3, C-4, and the carbon atoms of the exocyclic methylene and the 4-carboxyl groups were in the same plane; the presence of a broad singlet (Tables 1 and 3) suggests that the carboxyl group is perpendicular to the plane of the thiazinyl ring, *i.e.*, pseudo-axial.

The appearance of a pair of doublets in the p.m.r. spectra of pyridine solutions of Δ²-cephalosporanic acids (II; R' = H) (Table 2) must be attributed to the formation of a pyridine salt, which causes side-chain interaction. Thus, the exocyclic methylene group of the 2'-thienylacetamido-analogue (II; R = AcO, R' = H, R'' = 2-thienyl), which gives a pair of doublets in pyridine solution, yields a singlet at 5.25 τ in acetone. Pyridine salts (Table 3) and pyridinium derivatives of Δ²-cephalosporanic acids (Table 4) in deuterium oxide, however, show broad singlets.

Side-chain protons. The methyl ester protons of both methyl Δ²- and Δ³-cephalosporanates in deuteriochloroform solution (Table 1) show a sharp three-proton singlet at 6.13—6.21 τ; the methyl and methylene protons for the corresponding ethyl esters yield three-proton triplets centred at 8.70 ($J = 7.5$ c./sec.) and two-proton quartets centred at 5.75—5.76 τ ($J = 7.5$ c./sec.), respectively. The methylene and phenyl protons for the phenylacetamido-groups of the esters give rise to sharp two- and five-proton singlets at 6.15—6.37 and 2.65—2.70 τ, respectively; the 2'-thienylacetamido-analogues show a two-proton singlet at 6.14 τ for the methylene group and a one-proton triplet at 2.72—2.73 and a two-proton doublet at 3.02—3.03 τ ($J = 3.5$ c./sec.) for the thiophen ring. Similar peaks are shown by the free acids and salts. The other p.m.r. signals accord, both in position and in intensity, with the proposed structures, and are listed together with their assignments in the Tables.

EXPERIMENTAL

The infrared spectra over the 4000—650 cm.⁻¹ region were recorded on a Perkin-Elmer model 21 spectrophotometer fitted with a sodium chloride prism. The cephalosporanate esters were examined as 1.0% w/v solutions in bromoform in 0.8 mm. cells and as Nujol mulls; the other analogues were studied as Nujol mulls and occasionally as either potassium chloride discs or as solutions in dimethyl sulphoxide. The spectra of a selection of these compounds have been allotted nos. 13,300 onwards in the D.M.S. Scheme (Butterworths Scientific Publications, London).

The p.m.r. spectra were measured at 38° on a Varian Associates A60 spectrometer at a sweep rate of 1 c./sec./sec. and were calibrated against either tetramethylsilane itself or, for deuterium oxide solutions, against *t*-butyl alcohol or sodium 3-(trimethylsilyl)propane-1-sulphonate⁸ (assumed to be 2 c./sec. below Me₄Si) used as internal standards; were the results referred to tetramethylsilane (Me₄Si = 10.0 τ). The cephalosporanic esters, salts, and acids were studied as 5—10% w/v solutions in deuteriochloroform, deuterium oxide, and pyridine, respectively. Certain

⁸ Tiers, Abstracts of the 137th Meeting, Amer. Chem. Soc., 1960, 17R; Tiers and Coon, *J. Org. Chem.*, 1961, **26**, 2097.

analogues were studied in acetone, acetonitrile, formic acid, trifluoroacetic acid, and dimethyl sulphoxide. Methyl 7-phenylacetamidocephalosporanate was further examined in benzonitrile, dimethylformamide, dimethylacetamide, and dioxan. The high-temperature p.m.r. measurements on methyl 7-phenylacetamidocephalosporanate in dimethyl sulphoxide and on 3,21-diacetoxy-17-hydroxy-5 α -pregnane-11,20-dione in benzonitrile were conducted on a Varian Associates A60 spectrometer fitted with a variable temperature probe.

The cephalosporin analogues and the penicillin esters were prepared by colleagues in these laboratories and had the physical properties recorded in the references listed in the Tables.

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